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EVALUATION OF THE RADIOPROTECTIVE ACTION OF CHEMICAL AGENTS

Kenneth P. DuBois, et al

Chicago University

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Report No. 4

Evaluation of the Radioprotective Action of Chemical Agents

Final Report

Kenneth P. DuBois, Florence K. Kinoshita, Vivian Plzak
and Wendell Wong

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Contract No. DA-49-193-MD-2729

The University of Chicago
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13. ABSTRACT

The objective of this program was to screen compounds for radioprotective activity and to obtain information on those compounds found to have consistent activity. DRF studies were performed on 28 compounds that consistently protected at least 33% of the mice exposed to an otherwise lethal dose of X-irradiation. The compounds were tested in combination with MEA, PAPP and both protective agents. Using cholinesterase and spleen weight changes as indices of injury, an effort was made to categorize the compounds as being protective primarily against intestinal or hematopoietic injury. Several agents from the WRAIR program were tested for protective activity against radiation injury to intestinal or hematopoietic tissue using the same indices. It was possible to select combinations protecting both tissues. To obtain information on the metabolic aspects of the WRAIR compounds an esterase inhibitor and a hepatic microsome enzyme inducer were given to mice. The toxicity and radioprotective activity of the compounds could be altered by alterations of the activity of metabolic enzymes. The protective activity of dithiothreitol was found to be due primarily to the dextro isomer. The toxicity of the WRAIR compounds alone and in combination with MEA and PAPP and with MEA alone was investigated. The WRAIR compounds are of relatively low toxicity to mice. Combination with both MEA and PAPP reduced the amount of the WRAIR compound that could be tolerated. Combination with MEA alone did not reveal additive toxicity, but did reveal some enhancement of the radioprotective effect.

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SUMMARY

During the first three years of this program 8,000 miscellaneous compounds were screened for radioprotective activity in mice given a lethal dose (750 r) of whole body X-irradiation. Twenty-eight of the compounds which consistently gave at least 33% survival against a lethal dose of X-irradiation were subjected to more intensive study during the past four years. Accurate LD_{50} and careful DRF values were determined. The radioprotective effect of the compounds was further studied by combining them with mercaptoethylamine (MEA), p-aminopropiophenone (PAPP) and both MEA and PAPP. Biochemical studies using these 28 compounds and intestinal cholinesterase activity and spleen weight changes as indices of protective activity indicated that these agents did not have selective protective action on either tissue.

Several radioprotective agents were made available by the Division of Medicinal Chemistry, WRAIR, synthesis program to this program to determine their activity in combination with other agents. Of the WRAIR compounds those with the greatest protective activity had an accelerative effect on the recovery of spleen weights to normal after irradiation. Using combinations of the compounds from the screening program and the WRAIR program it was possible to select effective combinations using cholinesterase as an index of intestinal injury and spleen weight as an index of hematopoietic injury.

A limited number of experiments on the metabolic aspects of the WRAIR compounds indicated that the toxicity and/or radioprotective activity of the compounds could be altered by inhibition of hydrolysis with an aliesterase inhibitor, EPN (ethyl p-nitrophenyl phenyl phosphonothioate) or by stimulation of metabolism with a hepatic microsome enzyme inducer, phenobarbital.

The protective activity of the isomers of dithiothreitol as reported by other investigators was investigated. Radioprotective activity was found with the d-isomer of dithiothreitol when it was given before X-irradiation. The l form and the oxidized forms were not radioprotective.

Toxicity studies on the WRAIR compounds revealed that they are of relatively low toxicity to mice. Combination of these compounds with both MEA and PAPP reduced the amount of the WRAIR agent that could be tolerated by mice. Combination with MEA alone revealed little additive toxicity and some enhancement of the radioprotective effect.

FORWARD

Period covered: January 1, 1971 through December 31, 1972 with background information on the work accomplished from April 1, 1965.

Animal Experimentation: In conducting the research described in this report the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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I. Introduction

The original objective of this program was to find new radioprotective chemical agents. During the first three years of the program a total of 8,000 miscellaneous compounds were screened against radiation lethality in mice given a lethal dose (750 r) of whole body x-radiation (1). Of the total number of compounds screened 489 or 6.11% protected 17% of a group of 6 mice, 157 or 1.96% protected 33% of the mice, and 82 or 1.02% protected more than 33% of the mice.

The protective agents from the screening program, particularly those that afforded protection to more than 33% of the mice in the initial screening trial, formed the basis for a smaller research effort during the past 4 years. The compounds from the screening program that gave the highest amount of protection were retested. Twenty-eight compounds that consistently provided at least 33% survival of mice given an otherwise lethal dose of X-ray were subjected to more intensive study (2). DRF values were determined for each of these compounds. The radioprotective activity of the compounds was further explored by combining them with mercaptoethylamine (MEA), p-aminopropiophenone (PAPP) and both MEA and PAPP. A group of compounds from the WRAIR drug development program with known radioprotective activity was made available for inclusion in this program. Various combinations of compounds from the screening program and the special WRAIR compounds were studied to find combinations with enhanced radioprotective activity. The measurements of radioprotective activity of these compounds in various combinations was rather extensive and they provided a considerable number of combinations with relatively high activity.

Following thorough studies on the radioprotective activity of the compounds from the screening program alone and in various combinations, emphasis in the program shifted to a limited effort dealing with factors that influence radioprotective activity, the site of the protective activity among susceptible tissues, and the toxicity of the compounds (3).

The present report briefly summarizes the work done in previous years of the contract and describes in greater detail research done since the last report.

II. Materials and Methods

Adult, male CF_1 mice obtained from the Carworth Farms facility in New City, New York were used for these studies. The mice were 47 days old on arrival and were kept under observation for at least one week before use. Until they were irradiated the mice were kept in stainless steel cages containing 20 mice per cage. The mice were fed Rockland Laboratory Chow and water containing hydrochloric acid

to control infections. They were kept in animal quarters maintained at a temperature of 80° F. ± 2° F. Periodic bacteriological tests were made on the spleens of irradiated mice to rule out the presence of Pseudomonas using glycerol broth as a culture media and chloroform extraction of the pigment.

The radiation exposures were administered with either a General Electric Maximar III or a Keleket Therapy Unit operated at 250 KVP and 15 ma. The added filtration consisted of 0.25 mm. of copper and 1 mm. of aluminum. The focal skin distance was 56 cm. but minor adjustments were made prior to each radiation exposure period so that the dose rate was 66.6 r/minute. This was checked before each set of exposures and periodically during the exposure period with 250 r Victoreen ionization chambers which had been compared with a Bureau of Standards calibrated cobalt 60 source.

In the screening program an approximate acute LD_{50} for each chemical agent was measured by giving small groups of mice increasing dosage levels of each compound intraperitoneally. The toxic symptoms were observed for several hours and the mortality was observed over a 7-day period. The toxicity data were used to select the maximum tolerated dose for the radiation protection experiments.

All of the chemical compounds were supplied by WRAIR and were stored at 45° F. to 50° F. until needed. Each of the chemicals was dissolved or suspended in a 0.1% carboxymethylcellulose solution by homogenization prior to administration. The pH of the homogenate was adjusted to approximately 7.0 with either 1N HCL or 1N NaOH when necessary.

The radiation screening studies were carried out by injecting six groups each containing six mice with the maximum tolerated dose and an additional six mice with one-half of the maximum tolerated dose of each chemical 15 minutes prior to a lethal (LD_{99}/30 days) dose (750 r) of whole body x-irradiation. The two groups plus four control mice, that received only the vehicle for the drugs, were irradiated simultaneously and caged together. The animals were observed daily for a period of 30 days or until all of the mice were dead. Groups of mice that exhibited early deaths within six days of the x-ray exposure were re-tested either at the same drug dosage or at a lower dose. Early deaths could be attributed to either chemical toxicity or a synergistic effect of the chemical and radiation. The mice were individually placed in plastic centrifuge tubes during the radiation exposure. These tubes in turn were positioned radially on a rotating turntable to insure an even distribution of the dose. The environmental temperature was maintained at approximately 76° during the radiation exposure.

The radiation protection data were key-punched onto IBM cards and run through a sorting and error program which used the 1401 facility at the computer center at this institution. The resulting information was taped and submitted periodically to WRAIR.

Prior to DRF studies on compounds from the screening program that showed protective activity and studies on special compounds from the WRAIR drug development program, it was desirable to have accurate toxicity data on each compound. A sufficiently large number of mice (usually 30 to 40) were used to obtain accurate dose-response data. The LD_{50} values were calculated by the method of Finney (4) programmed for the IBM 7094 computer by Oldfield et al. (5). For the DRF studies at least 40 mice were used for each determination. In each instance the optimum time of administration, which had been previously determined, and approximately 2/3 of the LD_{50} (mg./kg.) of the drug were used. Varying x-ray doses were given and the resultant mortality was recorded for a period of 30 days or until all of the mice were dead. For the x-ray LD_{50} determination the same method was used as for the toxicity LD_{50} values for the chemicals. For the combination studies approximately 1/3 of the LD_{50} of a drug was given together with either 150 mg./kg. MEA, 20 mg./kg. PAPP, or 150 mg./kg. MEA and 20 mg./kg. of PAPP.

Some experiments were done to determine the type of protection afforded by the various chemicals. For these measurements cholinesterase activity (6) of the intestine was used to measure protection. For these measurements groups of at least 4 mice were pretreated with the drug, exposed to 800 r and sacrificed 3 days later. Spleen weight measurements were made on mice given 400 r of x-ray 2 days earlier and compared with irradiated and unirradiated controls.

The influence of drugs that induce increased activity of hepatic microsomal enzymes on the toxicity and radioprotective activity of the special WRAIR compounds was studied in mice. Microsomal enzyme assays were conducted using methods previously developed in this laboratory (7). The two microsomal enzyme assays were conducted using methods previously developed in this laboratory (7). The two microsomal enzyme systems employed were the phosphorothioate detoxification system and O-demethylase. Both of these enzymes respond to known inducing agents. Aliesterase assays were done using diethylsuccinate and tributyrin as substrates by the manometric procedure of DuBois et al. (8). Alpha-ketoglutarate oxidase was measured by the procedure of Ackermann(9).

III. Results

Radioprotective agents found during the screening of miscellaneous chemicals. The initial three years of this program were devoted exclusively to the screening of a wide variety of miscellaneous chemical agents for radioprotective activity. The data obtained from the

screening of 8,000 compounds were key-punched onto IBM cards and the information was taped and submitted to WRAIR.

The overall total number of compounds exhibiting some protection was 728 of the 8,000 that were tested which represented a total percentage of 9.09%. Most of the compounds (489) provided protection of only 1 of 6 mice against lethality from 750 r of x-ray. Protection at the level of 33.3% (2 of 6) mice was obtained with 157 or 1.96% of the compounds and 82 chemicals or 1.02% protected more than 33% of the animals.

All of the compounds that protected at 750 r were re-tested at the same x-ray dosage level. If protection was obtained again they were tested at 800 r. Of the 82 compounds that protected mice consistently at 750 r only 28 of them were also protective at 800 r. These 28 compounds were subjected to DRF studies and measurements of their protective activity in combination with standard protective agents. The best protective agents from the screening program thus served as the basis for other aspects of the program.

Acute intraperitoneal toxicity of radioprotective agents from the screening studies. In order to proceed logically with a study of the protective activity of the best compounds from the screening program it was advisable to obtain more accurate toxicity values for the compounds.

Table 1 summarizes the toxicity of the best protective agents from the screening program to mice.

Table 1

Acute Intraperitoneal Toxicity of Radioprotective Compounds from the Screening Program to Mice

WR No.	Chemical Name	No. of Mice	$LD_{50} \pm$ S.E. (mg./kg.)
P 5159	3-propionylindole	40	1436.4 ± 99.5
H 3820	2-hydroxy-5-methylaniline	40	270.7 ± 65.8
A 7130	ortho-aminophenol	41	623.6 ± 25.5
D 19860	2,5-dinitrophenol	41	379.0 ± 60.9
H 1295	4-hexen-3-ol	40	467.7 ± 86.8
H 5145	3-methyl indole	40	2140.2 ±102.6
N 2830	3-(omega-nitrovinyl)-indole	41	3291.1 ±129.0

Table 1 cont.

WR No.	Chemical Name	No. of Mice	$LD_{50} \pm$ S. E. (mg./kg.)
13415 C	2,6-diamino pyridine monohydrochloride	43	132.2 ± 5.0
19428A	N-benzoylphenyl hydroxylamine	55	421.4 ± 49.7
361 M	2-aminoethanethiol sulfuric acid sodium salt	40	1103.9 ± 42.8
5441 A	N-lauryl sulfolone-3-sulfonate	42	408.8 ± 29.6
6040 C	3,4-dibromosulfolone	35	459.4 ± 11.3
9217 A	N-sulfinyl-p-anisidine	36	462.3 ± 37.3
59082A	p-aminophenyl trifluoromethyl ether	39	50.2 ± 4.8
707 B	4-amino-2,1,3-benzothiodiazole	46	325.8 ± 28.5
4150 B	barium undecylenate	41	171.4 ± 6.4
872 E	barium-d-gluconate	39	124.0 ± 13.3
3966 D	diphenyldisulfide-2,2'-dicarboxylic acid	48	1849.8 ± 69.4
6431 B	benzolazine	41	1532.0 ± 41.3
14684B	4-hydroxy-3-methoxy benzylamino hydrochloride	36	1390.9 ± 24.2
16059 C	di-n-butyl carbinol	40	1206.4 ±110.4
12962 D	5-chloro-2-hydroxy aniline	36	252.1 ± 10.2
24024A	3-napthylmethyl-1'-imidazoline nitrate	36	61.4 ± 8.0
28470 A	bis(diglyme)sodium hexacarbonyl vanadate	34	44.4 ± 2.4
2822 D	S-2-(4-aminobutylamino) ethyl phosphorothioic acid	34	384.7 ± 14.6
2823 B	S-2(5-aminopentylamino) ethyl phosphorothioic acid	35	303.7 ± 23.2

Table 1 cont.

WR No.	Chemical Name	No. of Mice	$LD_{50} \pm$ S.E. (mg./kg.)
414 A	diethylammonium diethyl-dithiocarbamate	42	999.9 ± 35.8
14451 B	monoethyl fumarate	38	494.3 ± 37.6

DRF values for compounds from screening studies. Following completion of the toxicity measurements the DRF values were determined on the compounds (2). Preliminary tests were done with each compound to determine the optimum time of administration before the radiation exposure and the maximum tolerated doses of the agents. The x-ray LD_{50} for the drug-treated animals divided by the LD_{50} for irradiated controls give the DRF for a particular compound. Table 2 shows the radioprotective activity of 27 compounds from the screening program.

Table 2

Radioprotective Activity of Chemical Compounds from Screening Studies

Compound No.	No. of Mice	Dose of Chemical (mg./kg.)	Time of admin. of chemical before x-ray (min.)	$LD_{50} \pm$ S.E. (roentgens)	DRF
Control	334	--	--	525.4 ± 7.8	--
P5159	75	800	15	580.3 ± 69.1	1.11
H3820	120	150	15	613.0 ± 55.4	1.22
A7130	112	600	30	645.1 ± 23.5	1.28
D19860	75	200	0	654.2 ± 23.1	1.25
H1295	48	600	30	562.2 ± 46.3	1.12
M5145	65	700	15	705.4 ± 34.1	1.34
N2830	80	300	15	674.3 ± 18.6	1.29
13415C	47	50	15	686.9 ± 46.4	1.31

Table 2 cont.

Compound No.	No. of Mice	Dose of chemical (mg./kg.)	Time of admin. of chemical before x-ray (min.)	$LD_{50} \pm$ S. E. (roentgens)	DRF
19428A	65	300	30	835.7 ± 73.8	1.59
414A	63	600	0	626.8 ± 33.9	1.20
361M	72	350	0	741.1 ± 30.2	1.41
5441A	42	100	30	545.9 ± 85.6	1.03
6040C	72	100	15	669.7 ± 18.3	1.28
9217A	64	400	0	665.5 ± 13.1	1.27
59082A	75	20	15	695.8 ± 17.7	1.33
707B	96	100	15	704.6 ± 19.3	1.34
4150B	67	75	0	660.9 ± 32.6	1.26
872E	57	100	30	664.4 ± 22.1	1.27
3966D	67	600	15	656.2 ± 14.2	1.25
6431B	69	400	15	655.4 ± 16.5	1.25
14684B	71	500	30	621.7 ± 14.2	1.18
160590	53	800	15	554.7 ± 86.7	1.07
12962D	69	125	0	787.9 ± 25.3	1.50
28470A	75	50	0	884.5 ± 31.8	1.69
24024A	64	75	15	790.8 ± 21.7	1.50
2822D	72	250	30	797.8 ± 33.2	1.52
2823B	72	200	30	782.1 ± 45.1	1.49

The data in Table 2 show that the protective activity of most of those compounds which was observed in the initial sdcreening tests was confirmed by the DRF studies. The highest DRF value obtained by the use of those compounds alone was 1.69 for bis(diglyme) sodium hexacarbonyl vanadate. The DRF values for 10 other compounds were over 1.30. Many different chemical classes were included among the compounds that provided substantial protection including indoles, anilines, phosphorothioates, phenols, sulfones, azoles, thiazoles, carbamates and two barium salts.

DRF values for compounds from radiation screening studies given in combination with MEA, PAPP, or MEA plus PAPP. Additional background information on the activity of compounds from the screening program was obtained by studying their protective activity in combination with standard radioprotective agents. Table 3 summarizes the radioprotective activity, expressed in terms of DRF values, for 24 compounds from the screening program in combination with MEA. In each instance the same optimal time for administration of the compound before x-ray was used. The MEA was administered between 10 and 15 minutes before x-ray exposure at 150 mg./kg. The doses of the other chemicals were approximately 1/3 of the LD_{50}.

Table 3

Radioprotective Activity of Combinations of Chemical Compounds from Screening Studies and MEA (150 mg./kg.)

Compound No.	No. of Mice	Dose of chemical (mg./kg.)	Time of admin. of chemical before x-ray (min.)	$LD_{50} \pm$ S.E. (roentgens)	DRF
Control	334	--	--	525.4 ± 7.8	--
MEA	61	150	15	695.1 ± 18.5	1.32
P5159	43	400	15	1022.4 ± 87.1	1.95
H3820	48	90	15	997.0 ± 31.5	1.90
D19860	41	125	0	1031.7 ± 65.2	1.97
H1295	48	300	30	830.6 ± 15.7	1.58
N2830	40	150	15	778.6 ± 23.3	1.48
19428A	45	140	30	944.4 ± 27.0	1.79
14451B	44	165	30	830.7 ± 1.58	1.58
414A	51	300	0	870.5 + 12.4	1.65

Table 3 cont.

Compound No.	No. of Mice	Dose of chemical (mg./kg.)	Time of admin. of chemical before x-ray (min.)	LD_{50} ± S.E. (roentgens)	DRF
5441A	48	50	30	700.3 ± 19.9	1.33
361M	48	175	0	924.9 ± 19.5	1.76
6040C	45	50	15	730.3 ± 16.7	1.39
9217A	46	150	0	885.8 ± 20.7	1.68
59082A	47	10	15	1154.0 ± 66.6	2.20
707B	62	50	15	938.2 ± 11.9	1.78
4150B	47	50	0	785.9 ± 18.3	1.49
872E	42	40	30	896.1 ± 27.6	1.70
3966D	44	300	15	789.8 ± 21.9	1.52
6431B	48	200	15	796.3 ± 22.4	1.51
16059C	52	400	15	824.9 ± 11.2	1.57
12962D	40	80	0	987.3 ± 54.5	1.88
28470A	48	15	0	720.2 ± 60.3	1.37
24024A	69	20	15	897.7 ± 8.4	1.71
2822D	47	125	30	903.4 ± 21.2	1.71
2823B	48	100	30	880.0 ± 17.3	1.68

The lowest DRF value obtained for the combination of chemicals was 1.33 and 19 of the compounds exhibited a DRF value greater than 1.50. The DRF for MEA alone at the reduced dosage level employed in these experiments was 1.32. Only three of the compounds with DRF values over 1.0 when tested alone did not provide more protection in combination with MEA than was obtained with MEA alone. DRF values of 1.7 or greater were obtained with 11 of the combinations. Five of these combinations were of particular interest. The DRF values for these compounds in combination with MEA were as follows: p-aminophenol trifluoromethyl ether 2.2, 2,5-dinitrophenol 1.97, 3-propionylindole 1.95, 2-hydroxymethyl aniline 1.90, and 5-chloro-2-hydroxy aniline 1.88.

Table 4 summarizes the radioprotective activity expressed in terms of DRF values for compounds given in combination with PAPP. The optimum time of administration of each drug as determined previously was used for these experiments. The PAPP was given at a dose of 20 mg./kg. 10 to 15 minutes before x-ray exposure. Approximately 1/3 of the LD_{50} of each drug from the screening program was used.

Table 4

Radioprotective Activity of Combinations of Chemical Compounds from Screening Studies and PAPP (20 mg./kg.)

Compound No.	No. of Mice	Dose of chemical (mg./kg.)	Time of admin. of chemical before x-ray (min.)	LD_{50} ± S.E. (roentgens)	DRF
Control	334	—	—	525.4 ± 7.8	—
PAPP	71	20	15	848.3 ± 17.4	1.61
T5159	47	400	15	848.7 ± 24.5	1.61
H3820	40	90	15	1073.1 ± 52.2	2.04
A7130	44	200	30	840.1 ± 14.9	1.60
D19860	46	125	0	999.4 ± 21.7	1.90
H1295	40	300	30	955.9 ± 27.5	1.82
M5145	42	350	15	839.3 ± 37.8	1.60
13415C	49	25	15	879.9 ± 32.8	1.68
19428A	40	140	30	821.9 ± 61.1	1.56
14451B	40	165	30	835.5 ± 22.7	1.59
414A	54	300	0	1097.9 ± 28.2	2.09
361M	40	175	0	952.0 ± 39.5	1.81
5441A	45	50	30	817.6 ± 27.2	1.56
59082A	44	10	15	994.4 ± 44.8	1.89
707B	40	50	15	1033.6 ± 50.2	1.97
4150B	46	50	0	843.1 ± 24.2	1.60

Table 4 cont.

Compound No.	No. of Mice	Dose of chemical (mg./kg.)	Time of admin. of chemical before x-ray (min.)	LD_{50} ± S.E. (roentgens)	DRF
872E	47	40	30	880.4 ± 20.4	1.67
3966D	46	300	15	870.8 ± 18.7	1.66
6431B	47	200	15	860.9 ± 17.1	1.63
16059C	46	400	15	820.0 ± 16.6	1.56
12962D	47	80	0	910.0 ± 48.2	1.73
28470A	40	15	0	924.6 ± 25.2	1.76
24024A	46	20	15	783.8 ± 24.4	1.49

Significant increases in the DRF values were obtained with several combinations of the compounds with PAPP. The best protection in terms of DRF values was as follows; diethylammonium diethyldithiocarbamate 2.09, 2-hydroxy-5-methyl aniline 2.04, 4-amino-2,1,3-benzothiadiazole 1.97, 2,5-dinitrophenol 1.90, and p-aminophenyl trifluoromethyl ether 1.89. A few other compounds gave DRF values that exceeded the value for PAPP alone.

Table 5 shows the results obtained with the chemicals from the screening program given at their respective optimum times and at 1/3 of the LD_{50} in combination with MEA (150 mg./kg.) given 15 minutes before x-ray and PAPP (20 mg./kg.) given 13 minutes before x-ray exposure.

Table 5

Radioprotective Activity of Combinations of Chemical Compounds from Screening Studies and MEA and PAPP

Compound No.	No. of Mice	Dose of chemical (mg./kg.)	Time of admin. of chemical before x-ray (min.)	LD_{50} ± S.E. (roentgens)	DRF
Control	334	--	--	525.4 ± 7.8	--
MEA and PAPP	79	150 20	15 15	1132.3 + 66.5	2.15
P5159	44	400	15	921.6 + 23.8	1.76

Table 5 cont.

Compound No.	No. of Mice	Dose of chemical (mg./kg.)	Time of Admin. of chemical before x-ray (min.)	$LD_{50} \pm S._eE.$	DRF
H3820	45	90	15	1227.4 ± 41.2	2.34
A7130	40	200	30	1107.1 ± 39.8	2.10
D19860	48	125	0	1328.5 ± 54.0	2.53
N2830	45	150	15	1153.7 ± 54.8	2.19
13415C	41	25	15	1176.3 ± 49.9	2.24
19428A	64	140	30	1272.1 ± 30.5	2.42
14451B	68	165	30	1185.7 ± 20.2	2.26
414A	51	300	0	1311.1 ± 12.1	2.49
361M	42	175	0	1041.9 ± 33.8	1.99
6040C	51	50	15	1268.0 ± 37.4	2.41
9217A	40	150	0	1047.3 ± 55.0	1.99
59082A	40	10	15	1063.7 ± 50.6	2.02
707B	40	50	15	954.1 ± 29.8	1.81
4150B	40	50	0	1121.1 ± 49.4	2.13
872E	42	40	30	1261.0 ± 48.1	2.40
3966D	42	300	15	993.5 ± 23.7	1.89
12962D	40	80	0	1026.5 ± 48.3	1.96
28470A	44	15	0	880.1 ± 28.0	1.67
24024A	48	20	15	914.9 ± 35.9	1.74
2822D	49	125	30	1718.9 ±121.7	3.27
2822D	65	125	30	1638.6 ± 62.5	3.12
2823D	47	100	30	1151.0 ± 80.0	2.19

In all instances the DRF values were greater than for MEA and PAPP (2.15) which was the control for this experiment. The greatest protective effect was obtained with S-2-(4-aminobutylamino)ethyl phosphorothioic acid in combination with MEA and PAPP. Two successive trials with this combination gave DRF values of 3.27 and 3.12.

Biochemical measurements of radiation protection to the spleen and intestine by protective agents from the screening program. The most desirable combinations of protective agents would consist of agents that protected different organ systems. An attempt was made in this program to obtain some direct information on the protective effects of various compounds on the spleen and intestine. Spleen weights were used as a measure of protection to that organ. Cholinesterase measurements on the jejunum could be used to measure intestinal injury because the cholinesterase activity is localized in the mucosa and the activity of the jejunum decreases when the mucosa is destroyed by radiation. Spleen weights were measured in mice at 2 days after 400 r and cholinesterase measurements were made at 3 days after 800 r. Most of the compounds afforded protection to the spleen and the intestine. An occasional compound protected one system to a significantly greater extent than the other system. N2830 [3-(omega-nitrovinyl)-indole] is an example of this effect since it provided much more protection to the intestine than the spleen. However, these measurements indicated that the compounds did not have a selective action on one or the other tissue. Compounds with a marked protective effect on the intestine, for example, could be used effectively in combinations since it is frequently the intestinal damage that limits the protective activity of chemical agents. An experiment was performed on the best combination (WR 2822, MEA, and PAPP) used in these studies. This combination appeared to provide more protection to the intestine than the spleen which probably explains its ability to protect against mortality at doses where intestinal injury prevents survival.

Protective effect of WRAIR compounds against hematopoietic injury as measured by spleen weights. Several of the best radioprotective agents from the WRAIR synthesis program were made available to this program to determine their effects in combination with other agents. Some of these studies have been summarized (3) in a previous summary report and will be described only briefly here.

Several of the WRAIR compounds were given to irradiated mice and the spleen weights were measured. The results of measurements of spleen weight at intervals during the first 6 days after irradiation indicated that the various protective agents had some influence on the amount of hemapoietic injury caused by radiation but the greatest effect was an acceleration of the rate of recovery of the spleen weight to normal. The compounds with the greatest radioprotective activity showed the most rapid return of spleen weights to normal.

Several of the WRAIR compounds were tested in combination with agents from the screening program. In the selection of combinations for this experiment an attempt was made to select agents that would provide more protection against both the hematopoietic and intestinal injury than was obtained with either agent alone. However, as indicated above there was no striking difference among any of the protective agents used in this program in their protective effects on the spleen and intestine. Table 6 summarizes the radioprotective activity of combinations of special WRAIR compounds and protective agents from the screening program.

Table 6

Radioprotective Activity of Combinations of Special WRAIR Compounds and Protective Agents from the Screening Program

WRAIR Special Compound No.	Dose (mg/kg.)	WRAIR Screening Compound No.	Dose (mg./kg.)	$LD_{50} \pm$ S.E. (r)	DRF
2721	500	H3820	90	788.3 ± 44.4	1.59
2721	250	707B	50	953.6 ± 56.9	1.93
2721	250	MEA + PAPP	150-20	1205.4 ±124.1	2.44
2529	500	MEA + PAPP	150-20	1073.3 ±109.3	2.17
2529	500	12962D	63	814.9 ± 25.3	1.65
2529	500	19428A	150	907.3 ± 58.1	1.83
2529	500	707B	50	900.0 ± 52.8	1.82
2823	100	19428A	150	709.3 ± 52.1	1.43
638	250	361M	175	800.0 ± 65.3	1.61
638	250	12962D	63	768.7 ± 52.4	1.55
638	250	872E	50	830.7 ± 37.4	1.68
638	250	59082A	10	989.3 ± 74.9	2.00
638	250	19428A	140	949.3 ± 52.0	1.92
44923	200	872E	50	808.2 ± 37.2	1.63
44923	200	4150B	37	781.4 ± 37.8	1.58
44923	200	13415C	25	868.8 ± 30.3	1.75
44923	200	19860D	100	862.5 ± 23.5	1.74
44923	200	28470A	15	1075.6 ± 56.4	2.18

The data in Table 6 show that several of the combinations of compounds from the screening program and special WRAIR compounds or standard radioprotective agents provide an appreciable amount of radioprotection. Thus when WR 2721 and WR 2529 were combined with MEA and PAPP the DRF values exceeded 2.0. When the phosphorothioate, WR 638, was combined with the fluorinated ether (59082A) the DRF was 2.0. Using cholinesterase measurements as a measure of intestinal injury and spleen weights as a measure of hematopoietic injury, the ether was more protective to the spleen and WR 638 was more protective to the intestine. Several of the other combinations provided more protection than was obtained with either compound alone. The results of this study demonstrated that it is

possible to select effective combinations using cholinesterase as a measure of intestinal injury and spleen weights as a measure of hematopoietic injury.

Factors affecting the radioprotective activity of WRAIR compounds. Some experiments were conducted to determine whether WRAIR protective agents undergo metabolic change prior to exerting their activity. It is known that the phosphorothioic acid derivatives and the sulfuric acid derivatives must be hydrolyzed to yield the free sulfhydryl group for radioprotective activity. However, there is no information concerning other metabolic changes that might affect activity. To obtain some information along this line experiments were conducted in which the activity of oxidative hepatic drug-metabolizing enzymes were altered by administration of an enzyme inducing agent (phenobarbital) or an esterase inhibitor. The esterase inhibitor used for this study was ethyl p-nitrophenyl phosphonothioate (EPN) which is a cholinesterase inhibitor. Mice were treated with phenobarbital (50 mg./kg./day) for 5 days. This treatment is known to cause marked induction of hepatic microsomal enzymes (10). At one day after the last dose of phenobarbital the toxicity of 14 special WRAIR compounds was measured. Similarly toxicity measurements were made on mice pretreated with EPN (4.8 mg./kg.) given in a single dose 24 hours earlier.

The toxicity of two of the compounds was substantially reduced by pretreatment of the mice with phenobarbital. They were thiosulfuric acid S-2-(4-(p-methoxyphenyl)-butyl)amino)ethyl ether (WR 3050) and N-decylaminoethanethiosulfuric acid (WR 1607). These two compounds are, therefore, detoxified by hepatic microsomal enzymes. Although some other members of the group may undergo metabolism catalyzed by these enzymes the toxicity of the end-product does not differ appreciably from that of the parent compound.

When mice were treated with an aliesterase inhibitor 24 hours before administration of the radioprotective agent the toxicity of WR 2691, 2496, 2950, and 1607 was rather markedly increased. A smaller increase in toxicity was observed with 361 K, 1618 D, 2347, 3050, and 2822. The results of these measurements indicated that the esterases that are inhibited by EPN probably function to hydrolyze the group that covers the sulfhydryl group of the protective agent. In those cases where the toxicity of the compound was increased by EPN it appears that the unhydrolyzed parent compound is more toxic than the aminothiol derivative.

Those agents whose toxicity was affected by phenobarbital or EPN were subjected to further tests to determine whether the radioprotective activity was also altered by the drug. The increased toxicity of WR 2496, WR 2347, and WR 1607 to EPN-treated mice necessitated reduction of the dose administered in the radioprotection studies as compared with controls. The radiation protection appeared to be the same as in controls given the same doses of the drugs.

However, if the apparent increased toxicity of radiation due to EPN treatment, as evidenced by a DRF of 0.76 is taken into consideration it may be concluded that the protective effect was greater than was obtained in controls given the same dose of radiation.

In mice pretreated with phenobarbital, the toxicity of WR 3050 was reduced but the radioprotective activity was the same as in the controls. The results obtained with WR 1607, whose toxicity was also reduced by phenobarbital, were similiar to those obtained with WR 3050 in that the radioprotective activity was no different from controls at an equivalent dose. Phenobarbital thus causes some metabolic change in these compounds in toxicity without a decrease in radioprotective activity.

Radioprotective activity of miscellaneous chemical agents. A minor aspect of this program has been to determine whether reports of radioprotective activity among various chemical agents can be verified under our conditions of measuring radioprotection. One interesting report (11) involved the radioprotective activity of dithiothreitol. Falconi et al. (11) reported that this compound had protective activity and that the oxidized form of this compound had higher protective activity than the reduced form. In a later report (12) these investigators presented evidence that reduced dithiothreitol (120 mg./kg.) increased survival from 5.6% to 29.0% after 625 r and that the same amount of protection could be obtained if the compound was given up to 24 hours after irradiation. The oxidized form at 200 mg./kg. exhibited greater protective activity (56% survival) than the reduced form when given before irradiation and 46% survival was obtained when the compound was given as late as 24 hours after irradiation. The postradiation protective effect, the greater protection by the oxidized form than the reduced form, and the absence of an amino group in the structure makes dithiothreitol unique among radioprotective agents.

To ascertain whether the protective effect of dithiothreitol could be confirmed under our experimental conditions, experiments were done in which the oxidized and reduced forms of the compound were given to mice. Dithiothreitol was kindly supplied by Dr. Marvin Carmack, Department of Chemistry, University of Indiana. The dextro and levo forms of dithiothreitol were supplied as well as the dextro and levo forms of 3,4-dihydroxy-1,2-dithiane which is the oxidation product derived from the two dithiols.

The acute toxicity of the five compounds to mice is summarized in Table 7 where it may be seen that the oxidized form of the optical isomers of dithiothreitol exhibit the least toxicity of the five compounds. It is of considerable interest that there is a significant difference between the toxicities of the d and l forms of dithiothreitol. The toxicity of the commercially available racemic mixture more closely resembled that of its more toxic enantiomeric component. When toxic doses of either the d or l forms of the compound were given the resulting symptoms were the same. They consisted of hyperexcitability progressing to convulsions when lethal doses were administered. Symptoms appeared within 10 minutes and death occurred within one hour after lethal doses.

Table 7

Acute Toxicity of Dithiothreitol and its Oxidation Product to Mice

Compound	No. of Mice	LD50 ± S.E. (mg./kg.)
Racemic dithiothreitol	70	169.0 ± 2.7
L-dithiothreitol	36	179.0 ± 4.2
D-dithiothreitol	54	254.9 ± 8.5
oxidized L-dithiothreitol	24	410.0 ± 20.2
oxidized D-dithiothreitol	20	435.0 ± 19.4

Investigation of the radioprotective effect of dithiothreitol showed that d form protected mice against an otherwise lethal dose of x-radiation. Maximal protection was achieved by administration of doses of 200 mg./kg. Significant protection could be obtained with a dose of 150 mg./kg. The protective effect of this isomer is summarized in Table 8.

Table 8

Radioprotective Activity of d-Dithiothreitol (DTT)

Dose of d-DTT (mg./kg.)	Time of DTT admin. before x-ray (min.)	Dose of x-ray (r)	Mortality	% Mortality
—	—	500	4/10	40
—	—	600	5/10	50
—	—	625	9/10	90
—	—	650	10/10	100
—	—	700	10/10	[illegible]
—	—	750	10/10	100
60	10	625	9/10	90
120	10	625	9/10	90
150	10	625	7/10	70
150	10	650	4/10	40
200	10	600	2/10	20
200	10	650	5/10	50
200	10	700	6/10	60
200	10	750	6/10	60

The higher toxicity of the l form of dithiothreitol prevented the administration of 200 mg./kg. The results obtained with this compound in irradiated mice are summarized in Table 9.

Table 9

Lack of Radioprotective Activity by L-Dithiothreitol (DTT)

Dose of L-DTT (mg./kg.)	Time of L-DTT admin. before x-ray (min.)	Dose of x-ray (r)	Mortality	% Mortality
120	10	625	9/10	90
150	10	625	9/10	90
150	10	650	10/10	100
150	10	700	10/10	100
150	10	750	10/10	100

The data in Table 9 show that l-dithiothreitol provides no protection against any of the dose levels of radiation administered when compared with controls.

Proof that the radioprotective activity of racemic dithiothreitol is accounted for by the action of only one of the enantiomers present might be obtained directly. Falconi et al.(11) studied racemic DTT at a dose of 120 mg./kg. Half of that dose should provide the protection attributable to one enantiomer. By administration of Dg-DTT at a dose of 60 mg./kg. with exposure to 625 r we hoped to compare our results with those of Falconi et al. (11). However, this low dose falls outside the dose range of protective activity using mortality as the end-point for Dg-DTT. Yet we showed that by using pure Dg-DTT, one removes the toxicity attributable to the Lg-DTT and can thus administer higher doses of Dg-DTT than of rac-DTT. This capability results in protective activity amounting to twice that obtained with rac-DTT against 625 r of x-radiation. Furthermore, against 750 r one can still achieve protection with Dg-DTT equal to the best obtained with rac-DTT against 625 r.

Falconi et al. (11) reported that the oxidized form of rac-DTT is more effective against 625 r of x-radiation than rac-DTT itself when the former was given in larger doses. We investigated

the radiation-protective activity of both oxidized Lg-DTT and oxidized Dg-DTT against 625-750 r of x-radiation. The results of these measurements are summarized in Table 10. We observed no protection by either oxidized enantiomer. Our irradiation conditions were admittedly harsh to probe the limits of the protection that could be obtained from the oxidized form of DTT and to eliminate the inconclusive results frequently obtained in radiation experiments at lower radiation doses with small numbers of animals.

Table 10

Radiation-Protective Activity of Oxidized Forms of DTT in Mice

Compound	Dose of compound (mg./kg.)[a]	Dose of x-ray (r)	Mortality	% Mortality
oxidized	200	750	10/10	100
Dg-DTT	300	625	10/10	100
	300	650	10/10	100
oxidized	150	750	10/10	100
Lg-DTT	200	750	10/10	100
	300	625	9/10	90
	300	650	10/10	100

[a]All drugs were administered 15 minutes before irradiation.

The most interesting data presented by Falconi and coworkers (11) concerned the ability of both oxidized and reduced rac-DTT to enhance the recovery of irradiated mice when the compounds are administered after irradiation (11). Our investigation of the recovery-enhancing effects of Dg-DTT and the two dithianes is summarized in Table 11. Our data at 625 r suggest that administration of these agents after irradiation does indeed enhance recovery. However, against 700 r or greater, Dg-DTT had no noticeable effect when administered after irradiation. Since Dg-DTT protected significantly against subsequent irradiation even at 750 r (Table 8) its radiation protective properties are of greater magnitude than its ability to enhance recovery.

Table 11

Recovery Enhancing Activity of Dg-DTT and Oxidized Forms of Lg-DTT and Dg-DTT Given to Mice after Irradiation

Compound	Dose of Compound (mg./kg.)[a]	Dose of x-ray (r)	Mortality	% Mortality
Dg-DTT	200	600	8/10	80
	200	625	7/10	70
	200	700	10/10	100
	200	750	10/10	100
Oxidized Lg-DTT	300	625	8/10	80
Oxidized Dg-DTT	300	625	8/10	80

[a]All doses were given 10 minutes after irradiation.

We also compared the effectiveness of Dg-DTT and mercaptoethylamine (MEA) as protective agents under strictly comparable conditions experimentally. An attempt was also made to ascertain whether MEA and Dg-DTT have additive protective effects. The results of these measurements are summarized in Table 12.

Table 12

Radioprotective Activity of MEA alone and in Combination With Dg-DTT in Mice

Dose of MEA (mg./kg.)	Dose of Dg-DTT (mg./kg.)[a]	Dose of x-ray (r)	Mortality	% Mortality
200	0	650	2/10	20
200	0	750	4/10	40
150	0	750	7/10	70
150	85	750	5/10	50

[a]Drugs administered 10 minutes before irradiation

Comparison of the protective activity of MEA with the results obtained with Dg-DTT (Table 8) showed that Dg-DTT is approximately equivalent in protective activity to MEA on a molar equivalent basis or two-thirds as active on a weight basis. When attempts were made to combine MEA and Dg-DTT, it was found that the toxicity is additive. It was necessary to reduce the dose of MEA to 150 mg./kg. plus 85 mg./kg. of Dg-DTT. The protective activity of this combination is greater than when MEA is given alone at 150 mg./kg. confirming the ability of Dg-DTT to protect mice against lethal levels of x-radiation.

These experiments on the radioprotective effect of dithiothreitol show the importance of stereochemistry as a variable affecting chemical radioprotection. The mechanism of action of dithiothreitol as a radioprotective agent has not yet been delineated. On considering the various mechanisms of action that have been proposed for protective thiols and in attempting to rationalize the difference in the protective activity of the Lg-DTT and Dg-DTT, no conclusion can be reached at this time regarding why one enantimer exhibits protective activity while the other does not.

It is possible that Lg-DDT has restricted access to the primary loci of action at which Dg-DTT affords its radiation protection. A restriction could be imposed upon the Lg-DTT molecule in one of several ways. Pharmacological inactivation could occur in the form of stereoselective nonspecific binding to plasma proteins or to cell-membrane or intracellular constituents. Lg-DTT would thus be unavailable to afford protection while Dg-DTT of opposite configuration and failing to meet a stereochemical requirement for binding would remain active. In their study of protection by optically active 2-aminobutylisothiourea dihydrobromide, Doherty and Shapira (13), using labeled enantimers to determine intracellular distribution, found significant differences in binding in the cellular fractions between the enantiomers.

<u>Toxicity of radioprotective agents from the WRAIR drug development program alone and in combination to rats and mice.</u> During the first year emphasis has been given in this program to measurements of the toxicity of a few of the best compounds from the WRAIR drug development program for radioprotective compounds. The compounds used for these measurements were WR 2721, WR 638, WR 2822, and WR 2823. MEA and PAPP were included because an ultimate goal was to determine the radioprotective activity that could be obtained with combinations of the radioprotective agents. The intraperitoneal toxicity of these compounds to mice is summarized in Table 13. Oral toxicity data on some of the compounds are also included.

Table 13

Acute Toxicity of WRAIR Protective Agents to Male Mice

Compound	Route	No. of Mice	$LD_{50} \pm S.E.$ (mg./kg.)
MEA	Ip	89	451.9 ± 14.3
WR 2721	Ip	69	1085.6 ± 63.4
WR 638	Ip	62	889.6 ± 44.8
WR 2822	Ip	74	573.3 ± 31.6
WR 2823	Ip	83	487.9 ± 11.1
MEA	oral	38	1919 ± 250
WR 2721	oral	50	1825 ± 175
WR 638	oral	45	2700 ± 300
WR 2822	oral	50	860 ± 75
WR 2823	oral	55	1430 ± 125

The five compounds listed in Table 13 did not exhibit high toxicity to mice by the intraperitoneal or oral routes. The least toxic of the compounds by the intraperitoneal route was WR 2721. The LD_{50} for this compound was nearly one gram per kg whereas the other three compounds (WR 638, WR 2822, and WR 2823) were about twice as toxic. The data obtained in this study provide a strict comparison of the toxicity of the compounds. Thus all compounds were dissolved in water immediately before administration and for the various doses volumes of solutions equivalent to 1% to 2% of the body weight were given. All mice were the same age (8 weeks) and they were housed under the same conditions with respect to diet and environmental temperature. All of the mice were observed for 30 days after treatment although deaths always occurred within 5 days when due to the drugs alone. By the oral route of administration the compounds were 2 to 4 times less toxic than when given intraperitoneally.

The different samples of some of the compounds that were on hand varied considerably in their toxicity to mice. The

LD_{50} of an old sample of WR 2822 was 3[illegible]1.6 mg./kg. whereas the values for new samples were 573 and 534 mg./kg. Different samples of WR 2721 and WR 638 did not show differences in toxicity. However, WR 2823 did show differences in that the LD_{50} for an old sample was 274 mg./kg. as compared with a value of 488 mg./kg. for a new sample (No. 87950). It seems probable that there was a difference in toxicity of these samples at the time of their arrival because they were all refrigerated under identical conditions after arrival.

Accurate LD_{50} values for these compounds were also determined in rats. The results of these measurements are summarized in Table 14.

Table 14

Acute Intraperitoneal LD_{50} of WRAIR Protective Compounds to Male Rats

Compound	No. of Rats	LD_{50} ± S. E. (mg./kg.)
MEA	24	226.4 ± 14.9
WR 2721	30	695.4 ± 36.8
WR 638	28	600.0 ± 25.0
WR 2822	24	261.6 ± 7.8
WR 2823	28	250.0 ± 18.0

A comparison of the data in Table 13 and 14 show that there are considerable differences between rats and mice in their susceptibility to the acute toxicity of all four of the WRAIR compounds and MEA. Rats were about twice as susceptible as mice.

Before radiation protection studies were carried out with combinations of these compounds, the toxicity of combinations of the agents was measured in mice. For these measurements groups of 10 mice were given 150 mg./kg. of MEA and 20 mg./kg. of PAPP intraperitoneally and this was followed immediately afterwards by various doses of the WRAIR protective agents. In this manner it was possible to tell whether the combination of the WRAIR agent and MEA and PAPP resulted in additive, more than additive, or less than additive toxicity. The interest in this type of experiment lies in the fact that combinations selected for practical use will probably always contain MEA because of its relatively low toxicity among synthetic thiol derivatives and its availability at a low expense.

Table 15 summarizes the results of toxicity tests on combinations of MEA, PAPP, and WRAIR compounds.

Table 15

Intraperitoneal Toxicity of WRAIR Compounds Alone and in Combination with MEA and PAPP to Mice

WRAIR Compound No.	Dose of WRAIR Compound (mg./kg.)	PAPP (mg./kg.)	MEA (mg./kg)	Mortality	% Mortality
2822	150	20	150	2/10	20
2822	200	20	150	4/10	40
2822	300	20	150	8/8	100
2822	400	20	150	9/9	100
2822	500	20	100	10/10	100
2823	150	20	150	1/10	10
2823	200	20	150	3/10	30
2823	250	20	150	5/10	50
2823	300	20	150	7/10	70
2823	400	20	150	10/10	100
638	200	20	150	1/10	10
638	250	20	150	5/10	50
638	300	20	150	9/10	90
638	400	20	150	8/8	100
2721	200	20	150	0/10	0
2721	250	20	150	3/10	30
2721	300	20	150	5/10	50
2721	400	20	150	8/10	80
2721	500	20	150	10/10	100

Combinations of the WRAIR protective agents with MEA and PAPP reduced the dose of the WRAIR protective agent that could be tolerated rather markedly. The results clearly indicated that not as much protective activity could be obtained with the combination of the three chemicals than if the toxicity was not additive.

A rather high fraction of the lethal dose of PAPP is always necessary to reduce radiation lethality. We were interested in measuring the amount of increase in toxicity of the WRAIR agent if the PAPP was omitted from the mixture. Table 16 shows the combined toxicity of MEA and the WRAIR compounds given intraperitoneally to mice.

A number of experiments conducted on irradiated mice showed that MEA and WR 2721 gave more protection than could be achieved by either agent alone. The amount of protection could be varied by changes in the concentrations of either protective agent. The higher the WR 2721 concentration was in the mixture, the lower was the toxicity to mice. Very similar results were obtained with MEA and WR 638.

Table 16

Intraperitoneal Toxicity of WRAIR Compounds and MEA to Mice

WRAIR Compound No.	Dose of WRAIR Compound (mg./kg.)	Dose of MEA (mg./kg.)	Mortality	% Mortality
2822	200	150	0/10	0
2822	300	150	0/10	0
2822	400	150	2/10	20
2822	500	150	0/10	0
2823	200	150	0/10	0
2823	300	150	0/10	0
2823	400	150	0/10	0
2823	500	150	0/10	0
2823	600	150	4/10	40
638	300	150	0/10	0
638	400	150	3/10	30
638	500	150	5/10	50
638	600	150	9/20	45
638	700	150	9/10	90
2721	700	150	0/10	0
2721	1000	150	0/6	0
2721	1300	150	2/6	33

The data in Table 16 clearly show that there is very little additive toxicity between MEA and the WRAIR protective compounds. Thus it is likely that these protective agents will give more radiation protection than can be achieved with either agent alone.

IV. Conclusions

This program was initiated to screen miscellaneous chemical compounds for radioprotective activity. The program was confined to screening until 8,000 compounds had gone through the tests. Of these 489 showed protective activity (17%) in groups of 6 mice given 750 r of x-ray. 157 or 1.96% protected 33% of the mice and 82 or 1.02% protected more than 33% of the mice.

The protective agents from the screening program that afforded protection to more than 33% in the initial screening trial, formed the basis for a smaller research effort during the past 4 years. The compounds from the screening program that consistently gave at least 33% survival (28 compounds) were subjected to more intensive study. Careful DRF values were determined for each of these compounds. The radioprotective activity of the compounds was further explored by combining them with mercaptoethylamine (MEA), p-aminopropiophenone (PAPP) and both MEA and PAPP. A group from the WRAIR synthesis program with known radioprotective activity were made available to us for inclusion in this study. Various combinations from the WRAIR program and our screening program were studied to find combinations with relatively high activity.

Following thorough studies on the radioprotective activity of the compounds from the screening program alone and in combination, emphasis in the program shifted to a limited effort dealing with factors that influence radioprotective activity among susceptible tissues, and the toxicity of the compounds (3).

It is anticipated that the best protective compounds from the screening program here as well as those from the WRAIR will be subjected to much more intensive study at some time in the future. It was, therefore, concluded that accurate toxicity data on the compounds should be on hand for at least one species. Compounds from the screening program in this laboratory varied widely in chemical structure and in acute toxicity. They offer a wide variety of possibilities for structure-activity and mechanism studies.

Maximum protection by each of the 28 best compounds from our screening program was determined after accurate LD_{50} values and the optimum time of administration were determined. When 24 compounds from our screening program were tested in combination with MEA, 20 of them produced more protection than when tested alone. The compounds from our screening program were next studied in combination with MEA and PAPP. In all instances the DRF values were greater than for MEA and PAPP (2.15) which was the control for this experiment. The greatest protective effect was obtained with MEA, PAPP, and WR 2822. WR 2822 is actually from the WRAIR screening effort and the combination gave DRF values of 3.27 and 3.12.

The radioprotective activity of aminothiol derivatives is usually limited to their ability to protect the hematopoietic tissues or intestine. Thus categorizing protective agents in this manner should greatly aid in developing effective combinations. In this study biochemical procedures were developed for measuring the protective activity of various drugs to the intestine (2,3) and spleen (3) of mice. Compounds available to the program were selected for protection of spleen or intestine and given in combinations of two. In these experiments the compounds capable of providing the best protection to the two systems provided the best survival for irradiated animals.

A limited amount of work was done on the effects of inhibition or stimulation of drug metabolism on the activity of radioprotective agents. Some of the protective agents undergo typical drug metabolism changes before or after exerting their protective activity. When we altered the activity of these enzymes the protective activity of the drugs was frequently altered.

A small fraction of the efforts of this program dealt with confirming reports from other laboratories on protective activity of chemical compounds. During this study a report by Falconi et al (11) came to our attention. These investigators reported that they obtained radioprotection when they gave dithiothreitol before or after radiation and in either the oxidized or reduced forms. We did observe that the reduced form of dithiothreitol given before radiation exposure provided some protective effect.

Toxicity studies with the WRAIR compounds revealed that they are of relatively low toxicity to mice, but that rats are about twice as susceptible. Combination of the WRAIR compounds with both MEA and PAPP markedly reduced the amount of WRAIR compound that could be tolerated by mice. Combinations of the WRAIR com-with MEA alone gave evidence of little additive toxicity. The protection that could be obtained with 2 of the WRAIR compounds (2721 and 638) in combination with MEA was greater than with either agent alone.

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